

Ruling out the last fiction about miction by introducing μ CT-based Video-CysTometry

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Aims

Bladder problems, the small or large they are, always have an important impact on people's lives and social activities. These problems can range from small discomfort to being part of a bigger clinical picture or syndrome, such as multiple sclerosis (MS). Patients with MS often have bladder problems, next to the more obvious and better known motoric problems they suffer from. The most common problems are an increase in frequency and urgency or even incontinence, but also difficulties to begin miction (also called 'micturition', the medical term for 'urination'), leakage, or a sensation of incomplete urination and retention may also appear. In the case of urine retention, secondary urinary infections are a common problem for these patients.

Independent of the cause, resolving bladder problems will always have a significant impact on improving a patients' quality of life and is one of the motivations behind the continuing efforts to improve our fundamental understanding of the physiology of the bladder and the process of micturition, thereby aiming at finding solutions to patients' problems. In urological research, the standard technique to investigate the process of micturition is cystometry [1]. Cystometry essentially measures the pressure in the bladder and is already for over forty years the standard tool of urologists to investigate the function of the bladder and miction in lab animals. During a cystometry, physiological fluid is infused in the bladder at a constant speed, while the activity of the bladder is constantly monitored (Figure 1). This technique yields information about many parameters, such as the frequency of bladder contractions, how high the maximum and minimum pressure in the bladder are, how many contractions lead to micturition and which contractions do not, etcetera.

A favorable aspect of using this technique in the lab, is that it is essentially the same as the technique that is used in the clinic to investigate the bladder function in patients. This makes the results more easily translatable between bench and bedside, and parallel findings easy to interpret.

Although cystometry provides urologists already for over four decades with very useful information about the hyperactivity or hypoactivity of the bladder in animal models, there are still crucial parameters missing to make the puzzle [2]. From cystometry alone, a urologist can not know how full or empty the bladder is after a micturition phase, nor can he know whether there is still some urine left in the bladder after micturition or does he have information about the sphincter or possible urine leakage. Moreover, it is not known whether the monitored contractions originated from the bladder or are artifacts associated with abdominal pressure, movement, or something else. In order to rule out the fiction about miction, we aimed to explore the potential of μ CT to resolve the last mysteries about miction, which has led us to validate and introduce μ CT-based cystometry as a novel technique in the field of urology, baptized 'Video-CysTometry'.

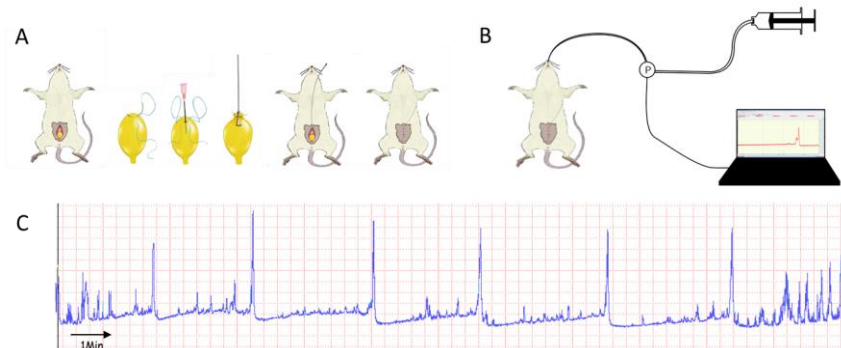


Figure 1: Standard cystometry. (A) A cannula containing a catheter and a pressure-sensitive probe is surgically inserted in the bladder of an anesthetized rat or mouse. (B) Through this cannula, physiological fluid is infused in the bladder at a constant speed while the pressure is monitored by the probe connected to the computer. (C) A typical pressure readout under constant infusion speed for a normal animal is shown, where peak pressures correspond to miction phases.

Method

Adult rats are kept under urethane anesthesia during the complete surgical and cystometry procedures. For classic cystometry, a cannula for constant infusion and probing the pressure in the bladder is surgically introduced in the bladder (Figure 1). The pressure in the bladder is constantly monitored over time. For μ CT-based cystometry, the infused physiological fluid was doped with a iodine-based contrast agent (Iomeron 350, Figure 2 panel A) at a concentration of 50 %, which had been optimized for viscosity and μ CT contrast generation. μ CT images were acquired on a dedicated small animal μ CT scanner (SkyScan 1076, Bruker microCT) using the following parameters: 50 kV, 0.5 mm Al filter, 200 μ A source current, 35 μ m isotropic resolution, 120 ms exposure time, 1 projection image per 1° rotation step and FOV covering the bladder. Infusion was paused until completion of the scan. A 'movie mode' acquisition of 2D projection images was acquired with similar settings to result in a frequency of 135 images per minute. The 'movie mode' acquisition, performed during constant bladder infusion, was validated as an accurate tool for real-time volume measurement by plotting the integrated pixel density of the bladder from the 2D movie, subtracting its background, and comparing the resulting images with the density of the 3D μ CT scan of the bladder, and this for different levels of bladder filling. The results were validated by scanning a known reference volume that was used as ground truth for the bladder volume calculations. As such, the relationship between the integrated pixel density from the 2D movie and the real-time volume of the bladder could be established, and hence used as measure of the volume of the bladder in real-time by acquiring a 2D-movie of the bladder dynamics. Next, this procedure was further optimized to obviate the need for 3D volume measurements by using the following protocol: for every animal, a baseline frame is acquired after which contrast infusion and Video-Cystometry are started. After acquisition, the integrated pixel density is measured at two points during filling of the bladder. The difference between those two values is compared with the infusion speed to yield the amount of added contrast per unit of time. Upon subtraction of the baseline image from the acquired movie, a new curve with absolute volume-measures can be calculated.

Results and discussion

μ CT was found to yield useful visualization of bladder filling and miction in both rats and mice infused with iodine-doped physiological fluid, without compromising standard cystometry due to altered viscosity of the fluid in the bladder. The μ CT data resulted in validated accurate measurements of bladder volume during the dynamic processes of bladder filling and emptying through miction phases. A high-frequency 2D movie-acquisition mode called 'Video-CysTometry' was optimized and validated to linearly correlate with the bladder contents, thereby enabling real-time volume measurements of the bladder that could be directly correlated to standard cystometry monitoring.

This novel approach resulted in the finding that normal, healthy (wild-type) mice do not empty their bladder completely after miction. The residue in the bladder after miction was found to be highly variable among wild-type mice. On the other hand, the maximum volume just before micturition was observed to be very constant. This is a remarkable finding as it is the contrary of what has always been assumed to be the case. Another new finding was that the residue after micturition strongly depended on the speed of filling of the bladder.

By comparing the pressure profile as measured by classic cystometry with the bladder volume as measured by Video-CysTometry, artefactual pressure changes could be isolated from relevant pressure changes originating from the bladder. Importantly, differences between bladder filling and residue can be huge among different animals (Figure 2 panel B), while classic cystometry traces and analytic results thereof resulted in almost identical measurements for these animals. These findings strongly indicate that Video-CysTometry results in more sensitive measurements of micturition than classic cystometry.

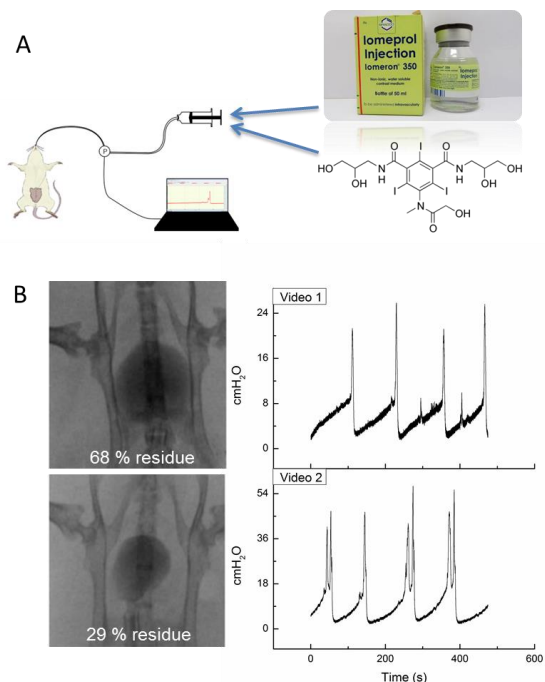


Figure 2: Video-CysTometry. (A) Iodine-based contrast agent is added to the physiological infusion fluid for enhancing μ CT contrast during Video-CysTometry. (B) Illustration of proof of concept: cystometry has always been regarded as a very sensitive tool for changes in bladder physiology. In these pictures, one can clearly see that while pressure traces are remarkably

similar (right column, classic cystometry), residual volume after a micturition cycle is completely different (left column, above compared to below, Video-CysTometry). This indicates that standard cystometry only hits the tip of the iceberg and that normal cystometry patterns do not necessarily indicate normal bladder function.

Conclusion

This is the first report showing the potential and power of the use of μ CT as a novel approach for cystometry. By dynamically visualizing the bladder contents during constant infusion, μ CT and fast 2D-acquisition enable the discovery of new findings and novel insights, unraveling the last mysteries of micturition and shifting longstanding paradigms in urology. μ CT is hereby established as a powerful novel tool that will potentiate our understanding of the fundamental physiology of micturition, as well as aid us in the search to better solutions to bladder problems related to diseases such as MS, ultimately aiming at finding solutions to improving the quality of daily life and easing the discomfort of many patients.

References

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